Studies of the Hydration and the Structure of Water and Their Roles in Protein Structure. III. Proton Magnetic Resonance Shifts of Water in Aqueous Solutions of Electrolytes and Their Relation to the Structure of Water

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In the preceding papers[1,2] of this series we reported on the states of water in aqueous electrolyte solutions and proposed that there are clathrates around various ions, perhaps forming an secondary hydration shell. In the present paper the effect of electrolytes and organic compounds on the structure of water, as estimated by measuring the chemical shift of the nuclear magnetic resonance frequency of water protons, will be described. It has been assumed that the number of hydrogen bond between water molecules decreases with an increase in the temperature and also that the structure of water is affected by such variables as the pressure and composition ot the solution. A proton magnetic resonance spectrometer is one of the most useful instruments for studying weak molecular interactions, particularly those of hydrogen bonds.[3]

Experimental

A JNM-3 high-resolution nuclear magnetic resonance spectrometer operating at 40 Mc./sec. in conjunction with an 12-in. magnet (Japan Electron Optics Laboratory Co.) was used. Proton magnetic resonance shifts relative to pure water were measured for the various salt solutions using the side-band method. The shifts observed for aqueous salt solutions were corrected for bulk diamagnetic susceptibilities. Acetone was found to be a suitable internal reference substance when corrections were made for the diamagnetic susceptibilities of salts, since the methyl protons of acetone are chemically unreactive and their resonance shifts are independent of the temperature. The procedure used was as follows: The same amount of acetone was added to the test salt solution and to the solvent used as an external reference. The relative shift of the methyl protons of acetone thus measured depends on the difference between the bulk diamagnetic susceptibility of the solution and that of the solvent. Therefore, it can be used to correct the observed proton magnetic resonance shifts of water protons.

All the solutions studied were prepared in a 0.01 M sodium ethylenediaminetetraacetate (EDTA) buffer and were adjusted to pH 7.0.

Measurements were made at 20°C.

Results

The relative shifts of the proton resonances of aqueous solutions containing various electrolytes or organic compounds are presented in Figs. 1, 2 and 3, where the shift, δ, is expressed in c. p. s. (at 40 Mc./sec.). A positive value indicates a shift to a higher resonance field than that of the external reference. Figure 1 shows the observed relative shifts of water protons (uncorrected for bulk diamagnetic susceptibilities) at various concentrations of salts. Figure 2 shows the corrected relative shifts for the bulk diamagnetic susceptibilities obtained by the measurement of the relative shift of the methyl protons of acetone. This correction is very important since the bulk diamagnetic susceptibilities of salt solutions are of the same order of magnitude or often even larger than the proton resonance shifts.

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[1] S. Goto, This Bulletin, 37, 1685 (1964).

[2] T. Isemura and S. Goto, ibid., 37, 1690 (1964).

[3] J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., N. Y. (1959).

[4] H. G. Hertz and W. Spalthoff, *Z. Elektrochem.*, 63, 1096 (1959).

These relative shifts were additive in all the salt solutions studied. Thus, as assumed by Hertz and Spalthoff,[4] if the relative shifts caused by potassium and by chloride ions in an aqueous potassium chloride solution are arbitrarily assumed to be equal, the relative shifts for each ion can be estimated. Figure 3 shows the dependence of the relative shifts for various ions on the concentration of the solution.

Discussion

The present paper will report on the effect of 1-1 electrolytes and organic compounds on the structures of water. Measurements were made of the shift of the nuclear magnetic resonance frequency of the protons of water molecules. At present a few reports[4-6] are available on the shift of water protons in aqueous solutions of various diamagnetic salts. However, in these reports the corrections of the diamagnetic susceptibilities of salts do not seem to be satisfactory. Shoolery and Alder,[5] for instance, did not make this correction.

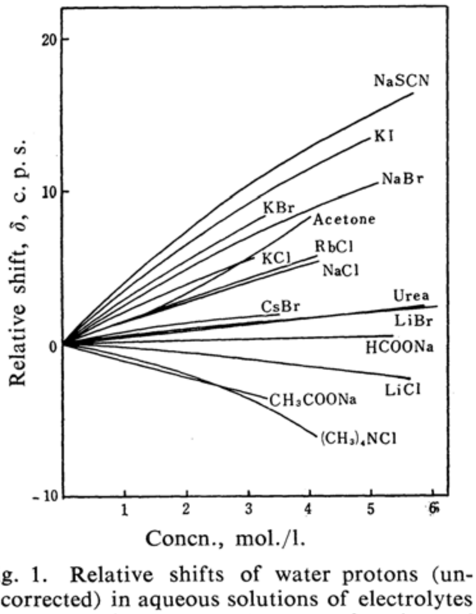

Fig. 1. Relative shifts of water protons (uncorrected) in aqueous solutions of electrolytes and organic compounds as a function of the concentration.

From Figs. 1 and 2 it can be seen that the relative shifts are in some cases as great as several cycles per second, which may be comparable with the correction values for ionic diamagnetic susceptibilities. Therefore, the shifts of water protons without correction for the bulk diamagnetic susceptibility do not show any real shift in aqueous salt solutions. Fabricand and Goldberg[6] have recommended the use of Pauling's values for the theoretical

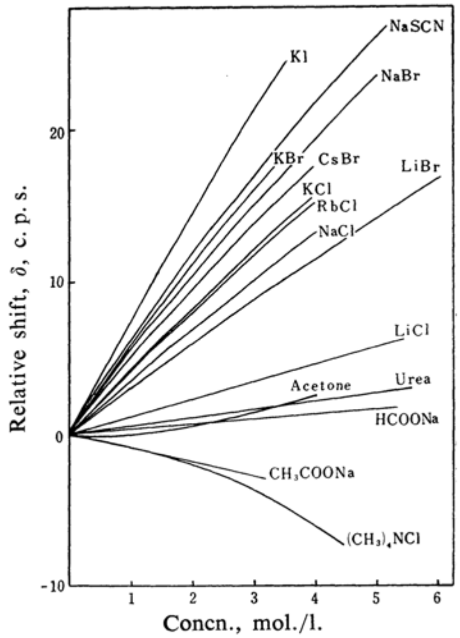

Fig. 2. Relative shifts of water protons (corrected for the bulk diamagnetic susceptibilities) in aqueous solutions of electrolytes and organic compounds as a function of the concentration.

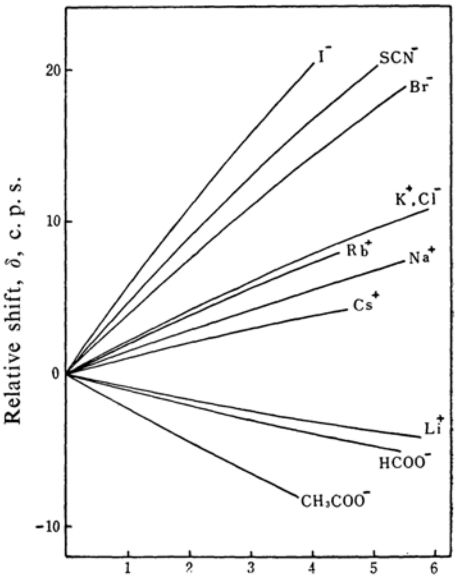

Fig. 3. Relative shifts of water protons for various ions as a function of the concentration (assuming that the relative shifts for potassium and chloride ions are equal).

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5) J. N. Shoolery and B. J. Alder, *J. Chem. Phys.*, **23**, 805 (1955).

6) B. P. Fabricand and S. Goldberg, ibid., **34**, 1624 (1961).

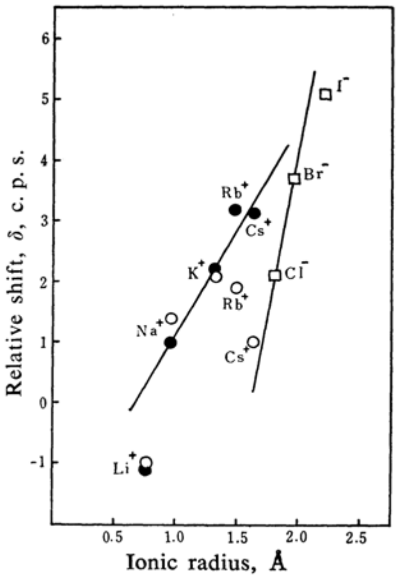

Fig. 4. Relative shifts of water protons for each ion at a concentration of 1 mol./l. as a function of the ionic crystal radius.

-●- Corrected from Pauling's theoretical diamagnetic susceptibilities.

-○,□- Obtained in this paper.

diamagnetic susceptibilities of crystals for this correction, because the relationship between the relative shifts, thus corrected, and the ionic crystal radius are linear. However, such a linear relation does not always hold true, since the behavior of water molecules in the presence of electrolytes is very complex, as has been described in the preceding papers.[1,2]

As has been mentioned above, acetone is a suitable homogeneous internal reference for studying the proton magnetic resonance of water. If corrections for the observed relative shifts of water protons are made using this method, no linear relation, such as that of Fabricand and Goldberg, holds any longer. This is shown in Fig. 4, which illustrates the relation between the ionic crystal radius and the relative shift of 1 M salts obtained from Fig. 3 and from calculations using Pauling's theoretical ionic diamagnetic susceptibilities for corrections. The straight lines are drawn in accordance with the values of Fabricand and Goldberg. The deviation from this linear relation is striking in the case of lithium and cesium ions. This will be discussed below.

Shoolery and Alder[5] interpreted the relative shifts of water protons in terms of the following two factors; (1) the ionic electric field distorts the polarization effect; and (2) the ion disrupts the structure of water and breaks the hydrogen bonds between water molecules. In the former case, the water molecule is polarized so that fewer electrons are found on the proton, which is therefore less shielded from the external magnetic field. On the other hand, in the latter case, the hydrogen bonds are generally broken by the ions. When a solute particle is pushed into a space between water molecules, the hydrogen bonds between water molecules are broken. Therefore, the proton is more shielded from the external magnetic field. This effect eventually counteracts the polarization effect on the proton.

As is shown in Figs. 2 and 3, in general, the resonance frequency of water protons in an inorganic salt solution is higher than that in pure water, and there is a tendency for a shift to a higher field to occur with an increase in the ionic radius. The direction of this shift agrees with that of the breakage of the hydrogen bonds. The results obtained in this experiment clearly demonstrate that the relative shift is more sensitive to the hydrogen bonds of water than the polarization effect.

Figure 3 shows the results obtained using the shift of the methyl protons of acetone for the correction of the diamagnetic susceptibility. It can be seen that the cesium ion is not so effective as the potassium ion in producing a water proton shift, although it has a larger ionic radius. This result agrees with those obtained by Hertz and Spalthoff[4] and Sato.*

It is generally believed that the number of the water hydrogen bonds decreases with an increase in the ionic radius.[7] In addition, the water molecules are thought to be less polarized when the ionic size is great. As a result of these effects, the proton is more shielded, and thus the relative shift to a high field becomes larger. In this respect, the behavior of the cesium ion is unusual. However, in the preceding papers of this series we proposed that around such ions as the cesium ion there is a clathrate which consists of water molecules mutually bound with hydrogen bonds. This conclusion is strongly supported by the present results, obtained by the method of nuclear magnetic resonance. This method is very sensitive to the state of hydrogen bonding, as will be described below.

In aqueous electrolyte solutions, the degree of hydration increases with a decrease in the ionic radius, as has been shown previously by studies of the ionic mobilities,[7] diffusion coefficients,[8] viscosity,[9] etc. However, the previous results were all based on various assumptions. Around ions there are not only water molecules of primary hydration but also

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* Personal communication.

7) R. W. Gurney, "Ionic Processes in Solution," McGraw-Hill Book Co., New York (1953).

8) J. H. Wang, *J. Phys. Chem.*, **58**, 686 (1954).

9) E. R. Nightingale, Jr., ibid., **63**, 1381 (1959).

of secondary hydration, the structures of which are different from that of pure water. The ions with strong electric fields, such as lithium, beryllium and magnesium, cause not only the contact of water molecules with the ions but also exert an orienting influence on more remote water molecules, resulting in a secondary hydration shell.[10,11]

Furthermore, in most electrolytes the water molecules in the first hydration shell probably exert an ordering effect upon the outer molecules by means of hydrogen bonding. This may also be inferred from a comparison of the behavior of anions and cations. As is shown in Fig. 4, the relative shifts of anions in relation to the ionic radius varies linearly. On the basis of viscosity studies,[9,10] anions such as chloride, bromide and iodide are thought to have little hydration, but to break the water structure. On the other hand, in the case of cations the relative shifts do not vary linearly with the ionic radius. The viscosity of an electrolyte solution referred to a cation is also complicated. These findings perhaps result from differences in the orientation of the water molecules to the ions.

Frank and Evans[12] suggested that an iceberg is formed around non-polar groups. Icebergs have also been demonstrated in gas hydrates,[13] proteins,[14-16] and molecular ions.[11,17,18] From studies of the partial molal heat capacities[11] and X-ray diffraction,[18] it has been suggested that the clathrates are formed around the tetraalkylammonium ions. Similarly, as is shown in Fig. 2, the resonance frequency of water protons affected by the tetramethylammonium ion shifts to a lower field than that in pure water. Compared with other ions, the number of hydrogen bondings of water molecules around the tetramethylammonium ion is probably large. That is to say, the shift is in agreement with the direction of the formation of hydrogen bonding, in spite of its larger size, which has a small polarization effect on water molecules. This result also supports the consideration that the shifts of water protons as a result of the presence of ions are mainly due to a change in the number of hydrogen bonds.

From these facts, it is likely that clathrates are formed around the cesium as well as the tetramethylammonium ion. This may be the reason why the increase in the relative shifts to higher fields is reduced in an aqueous cesium salt solution. In the case of the cesium ion, however, the increase in the number of hydrogen bonds is not greater than the decrease in the number of hydrogen bonds broken, since the relative shift is still positive.

On the other hand, another mechanism must be considered for the lithium ion, which has a smaller ionic radius and exerts a negative shift on the proton resonance. The lithium ion has a larger polarization intensity and exerts an orientating influence not only on the neighboring water molecules but also on remote water molecules. It is known that the lithium ion has a larger effective radius in viscosity.[9] The fact that the relative shift of the lithium ion is found at a lower resonance frequency than that deduced from the linear relation with the ionic crystal radius may be explained as follows: the many water molecules orientated to the ion change the relative shift to a lower field, and water molecules outside the orientated water molecule may induce the formation of hydrogen bondings between water molecules.

Thus, each of these ions, as has been mentioned above, would contribute to the denaturation of proteins and nucleic acids. For example, as there are two kinds of hydration, one of which is orientated to the polar groups of proteins and the other orientated as an iceberg or a clathrate around the non-polar groups of the protein, both these hydrations may be important in maintaining the secondary and tertiary structures of protein. Thus we must consider the actions of ions as protein-denaturing agents in connection with breakers and inducers of the water structure. Details of this will be discussed in the following paper.

Similarly, as may be seen from Fig. 3, formate and acetate ions also form a water structure with increased hydrogen bonds. The acetate ion has more ability to form hydrogen bondings than the formate ion. The methyl group of acetate may cause the formation of clathrates.

Furthermore, as is shown in Fig. 2, urea caused a slight breakage of the water structure. However, as urea has hydrophilic groups, water molecules are attracted to the urea molecule. There may be two kinds of water molecules around the urea molecule: water molecules with broken hydrogen bonds, and those with more than normal hydrogen bonding. This mechanism may resemble that of the large ions, such as cesium or formate. The number of these broken and formed hydrogen bonds may

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10) M. Kaminsky, *Discussions Faraday Soc.*, **24**, 171 (1957).

11) H. S. Frank and W. Y. Wen, ibid., **24**, 133 (1957).

12) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

13) W. F. Claussen, ibid., **19**, 259, 1425 (1951).

14) I. M. Klotz, *Science*, **128**, 815 (1958).

15) W. Kauzmann, *Advances in Protein Chem.*, **14**, 1 (1959).

16) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

17) F. S. Feates and D. J. G. Ives, *J. Chem. Soc.*, **1956**, 2798.

18) D. Feil and G. A. Jeffrey, *J. Chem. Phys.*, **35**, 1863 (1961).

be nearly the same. Therefore, it appears from these properties of urea that the phenomenon of the urea denaturation of proteins is complicated by such variables as the concentration of urea, the temperature and the kinds of substance.[19-21]

Summary

The effects of various electrolytes and organic compounds on the structure of water, and particularly on hydrogen bonding, have been investigated by measuring the chemical shift of water protons in the nuclear magnetic resonance frequency. The chemical shift observed for salt solutions has been corrected for the bulk diamagnetic susceptibility, using methyl protons of acetone as a reference. The chemical shifts of water protons in aqueous solutions of various solutes have been considered.

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20) M. Levy and J. P. Magoulas, *Fed. Proc.*, **20**, 381 (1961); **21**, 405 (1962).

21) W. Bruning and A. Holtzer, *J. Am. Chem. Soc.*, **83**, 4865 (1961).